Biodosimetry for long-term low-dose past radiation exposure
and
What is the lowest detectable dose?

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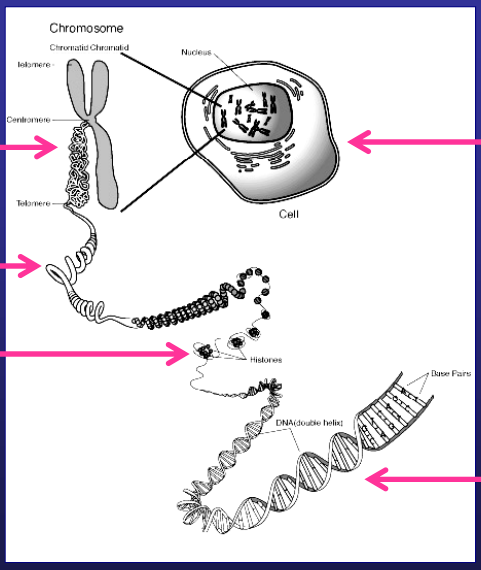
Outline of this Talk

- Chromosome aberrations
  - unbanded
  - banded - karyotyping
  - painting
- Dosimetry confounders – lessons learned
- Biodosimetry and translocation persistence
- What is the lowest detectable dose?

Cell and Chromosome Biology



Chromosome

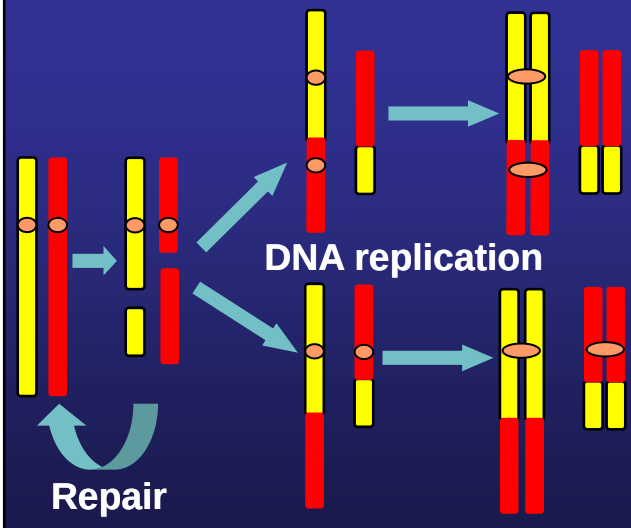
Cell

Genes

Histones

DNA

Chromosome Aberrations: Translocations and Dicentrics



Dicentric plus fragment (*lethal, low risk*)

DNA replication

Reciprocal translocation (*viable ==> RISK!*)

Repair

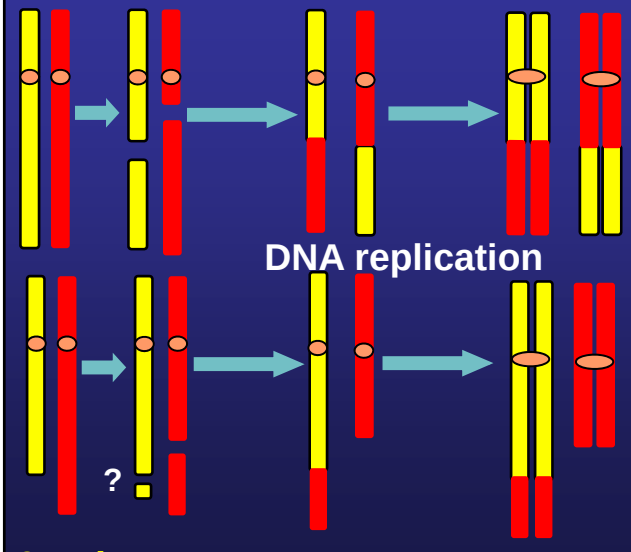
G1 phase

G2 phase, metaphase

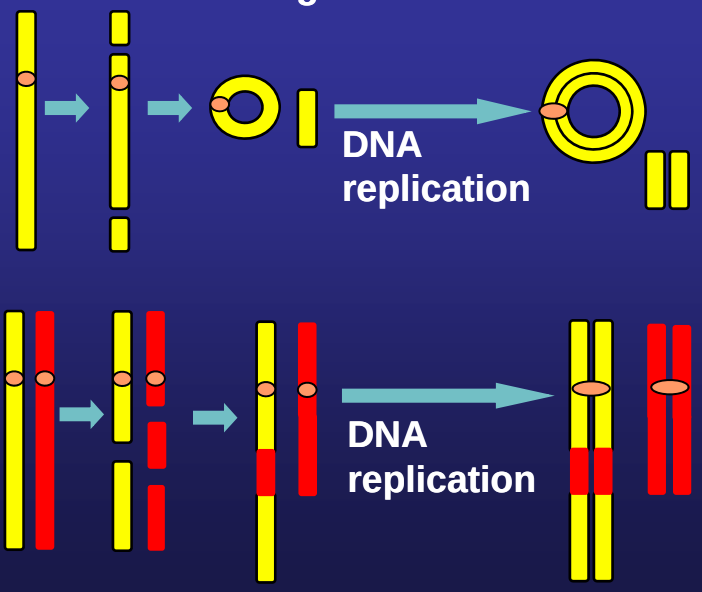
Characteristics of Translocations

- Induced at frequencies equal to dicentrics
- Stable through cell division
  - persist *in vivo* indefinitely
  - whereas dicentrics disappear rapidly
- Dosimetry for acute exposure is known
- Accumulate with chronic exposure
- Ideal for biodosimetry ("Gold Standard")
  - chronic exposure
  - exposure occurred many years previously

Chromosome Aberrations: Reciprocal and Non-Reciprocal Translocations

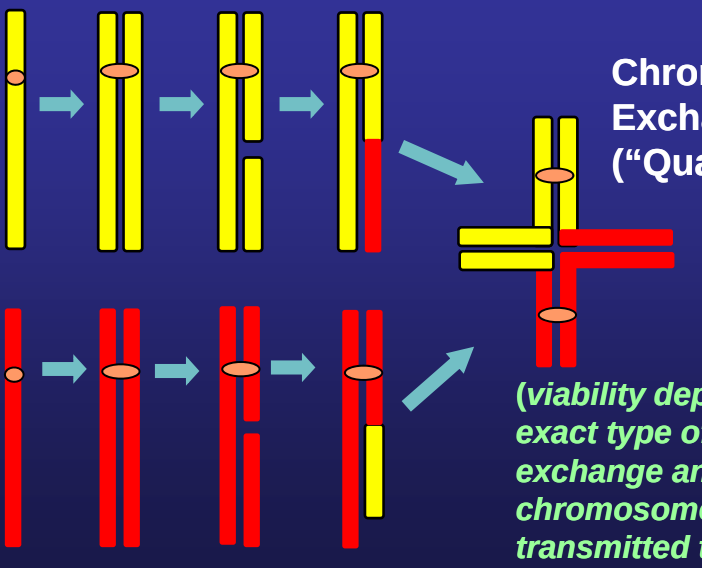


Chromosome Aberrations: Rings and Insertions



7

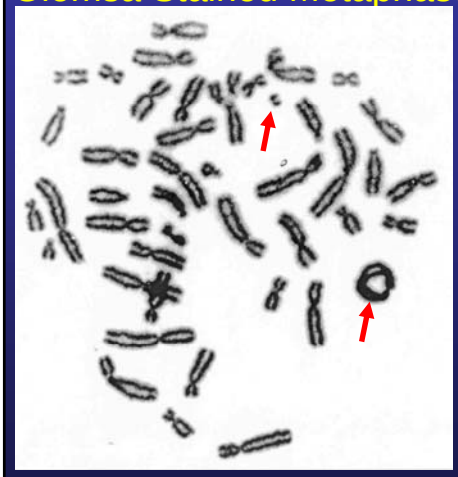
Chromatid Aberrations - One Example



8

Unbanded Chromosomes

Giemsa Stained Metaphase



- Detects “unstable” events
- Used widely in research
- Moderate analysis speed
- Inexpensive reagents

Chromosome Aberrations - Unbanded

Human cell in metaphase stained with Giemsa

With conventional stains, some categories of chromosome aberrations cannot be seen.

Fragments - yes

Dicentrics - yes

Chromatid damage - yes

Translocations - generally no

Insertions - no

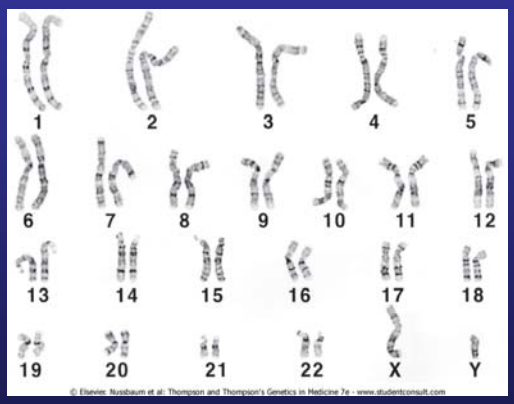
Complex rearrangements - no

Resolution is limited!

Karyotyping

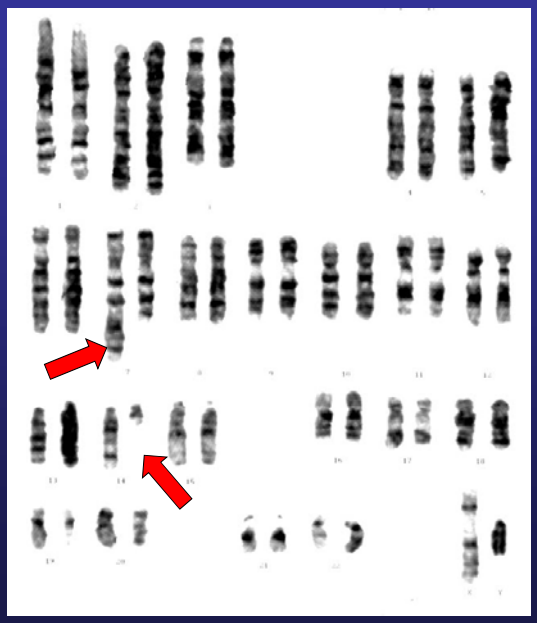
Giemsa Banding

Metaphase



Karyotype

Karyotype with a translocation



Slow and expensive!

Karyotyping

With chromosome banding, all categories of chromosome aberrations can be seen.

Fragments - yes

Dicentrics - yes

Chromatid damage - yes

Translocations - yes

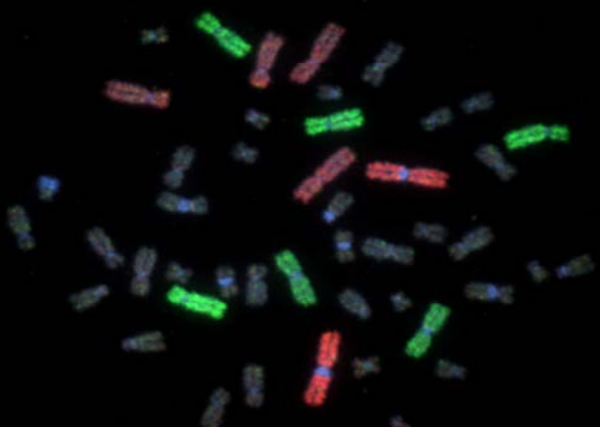
Insertions - yes

Complex rearrangements - yes

Speed is limited!

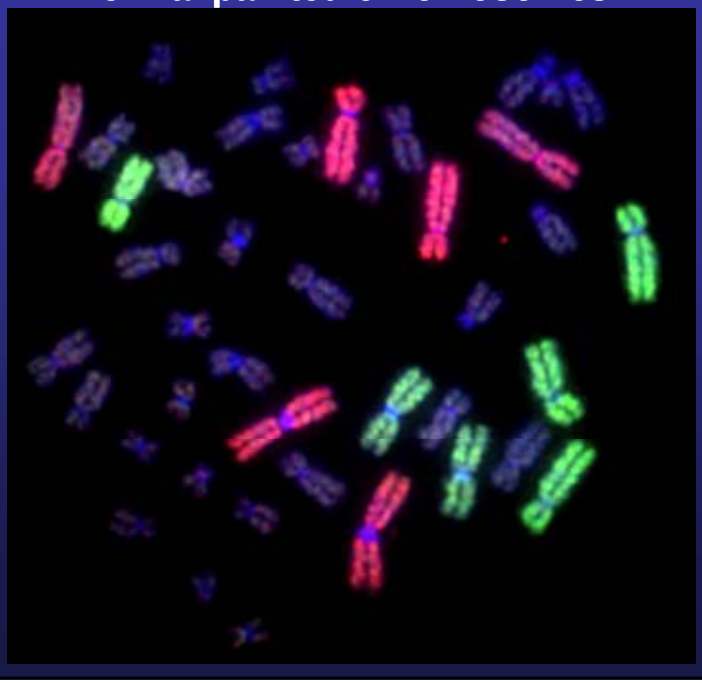
Human Chromosome Painting

Normal chromosomes



Chromosomes 1, 2, and 4 are painted red; 3, 5, and 6 are painted green.

Normal painted chromosomes



What is the difference between FISH and Chromosome Painting?

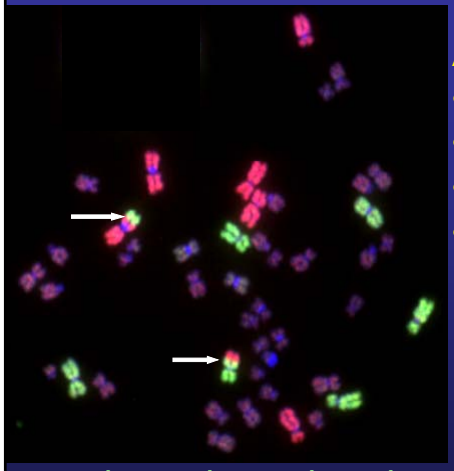
FISH: fluorescence *in situ* hybridization

Chromosome painting: one of many applications of FISH

Not all chromosome painting is done by FISH

Not all FISH is chromosome painting

Translocations by Painting



Advantages for biodosimetry:
- speed (color junctions)
- sensitivity
- reliability
- relevance

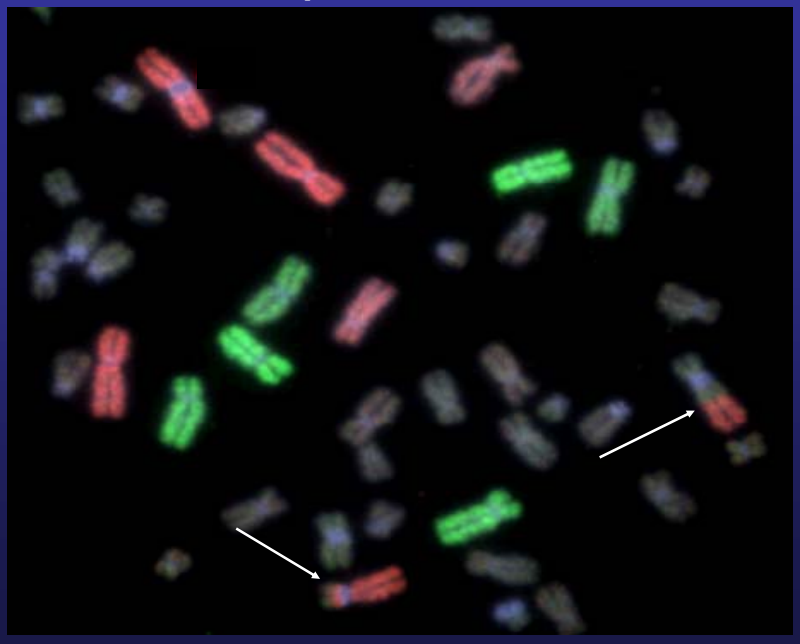
Painting detects:
- color “junctions”
- breaks / fragments

BUT: Not every aberration is detected!

Reciprocal translocations are the hallmark of ionizing radiation exposure

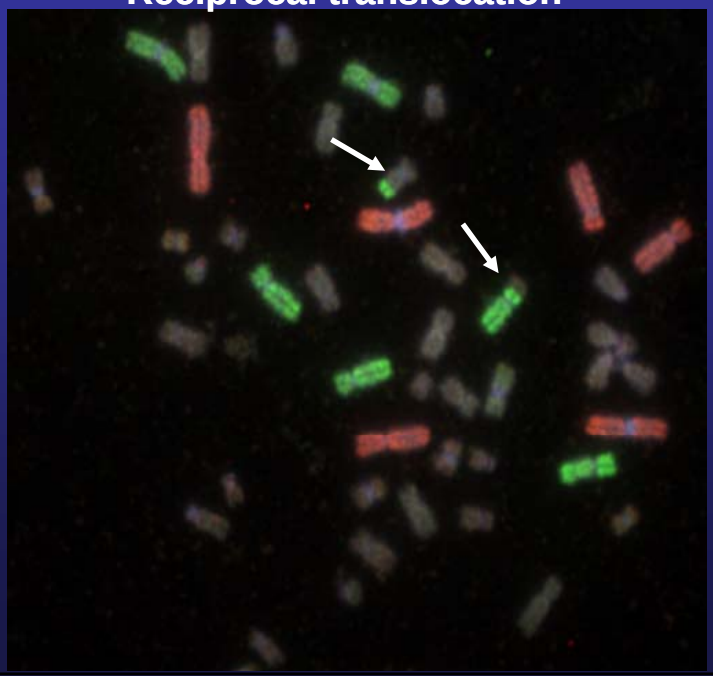
17

Reciprocal translocation



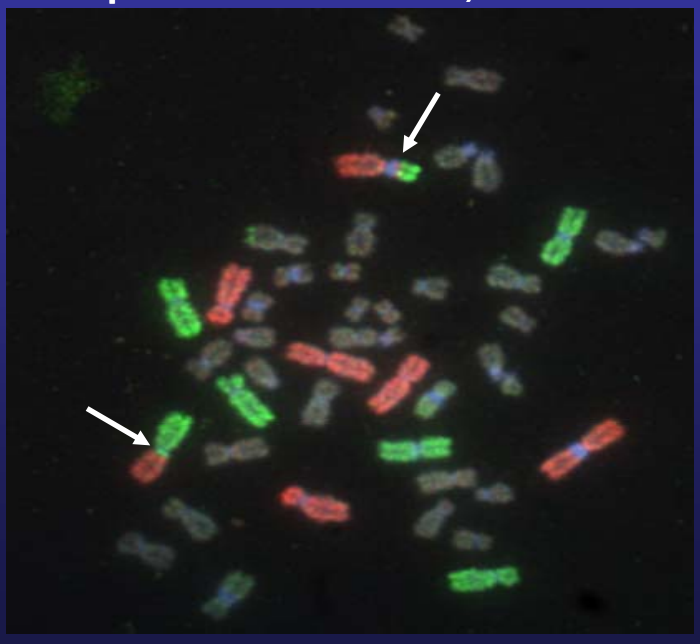
18

Reciprocal translocation



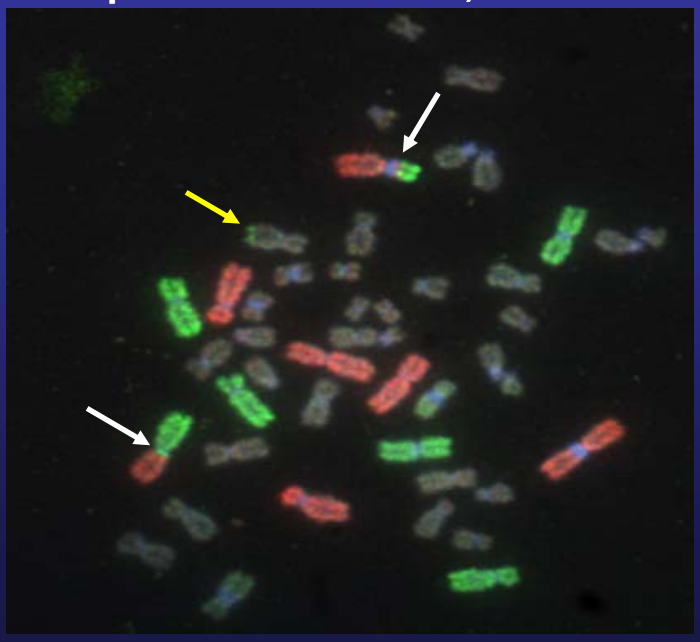
19

Reciprocal translocation, and . . . ?

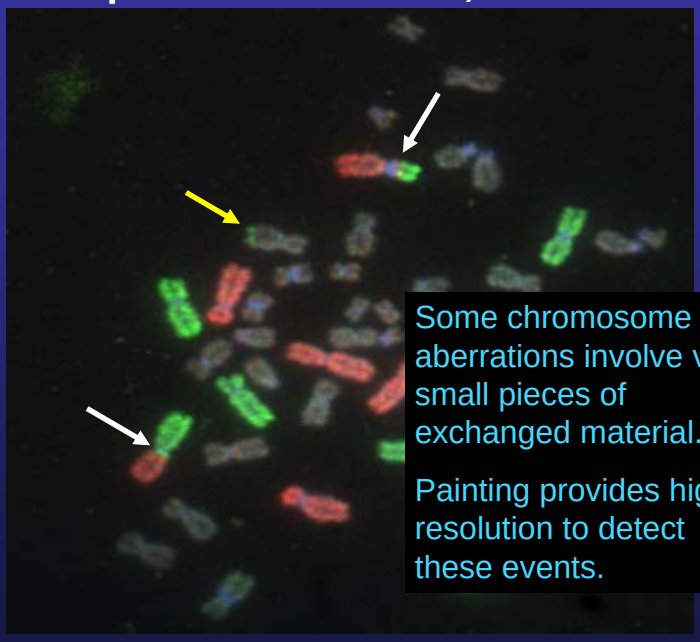


20

Reciprocal translocation, and . . . ?



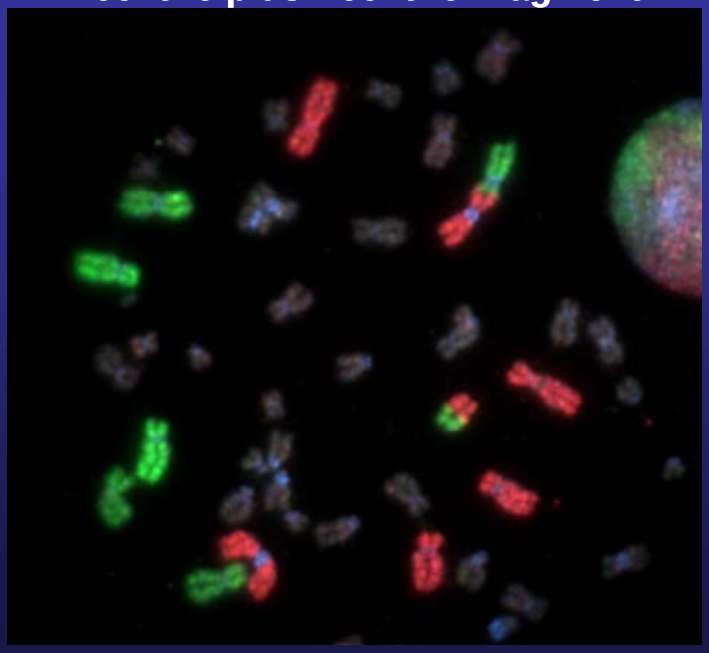
Reciprocal translocation, and . . . ?



Some chromosome aberrations involve very small pieces of exchanged material.

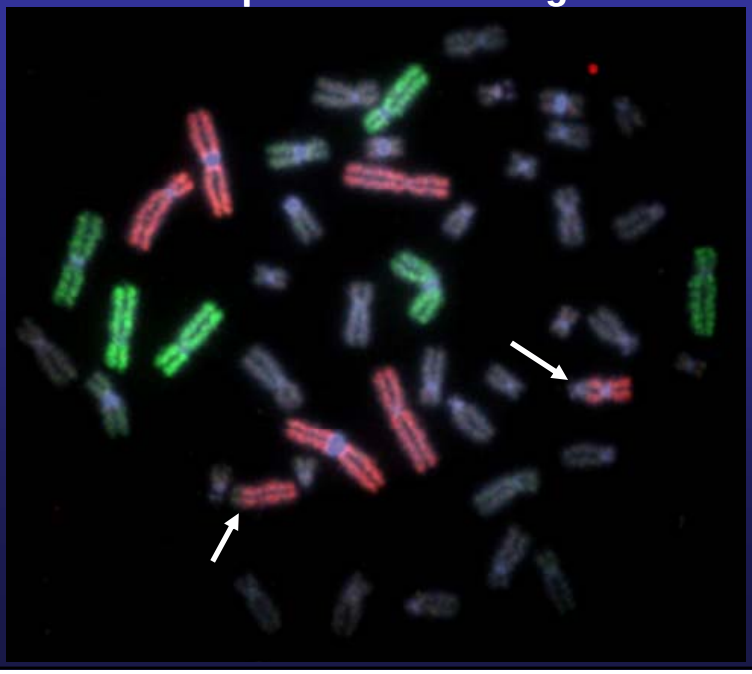
Painting provides high resolution to detect these events.

Dicentric plus Acentric Fragment



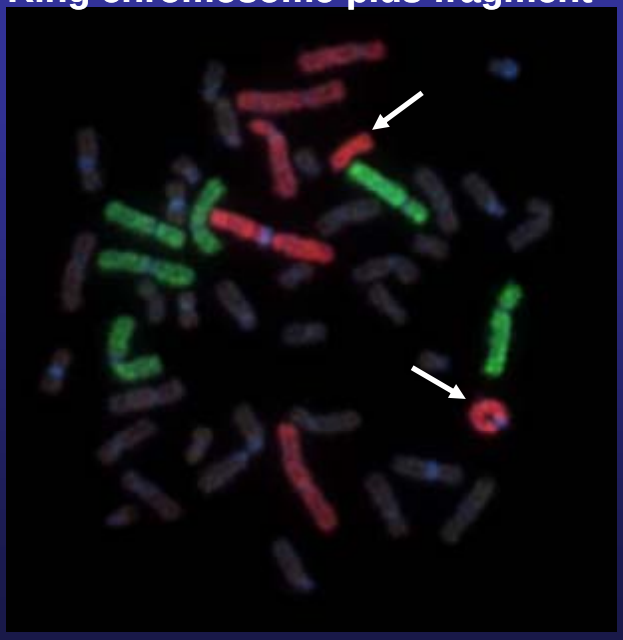
23

Dicentric plus Acentric Fragment

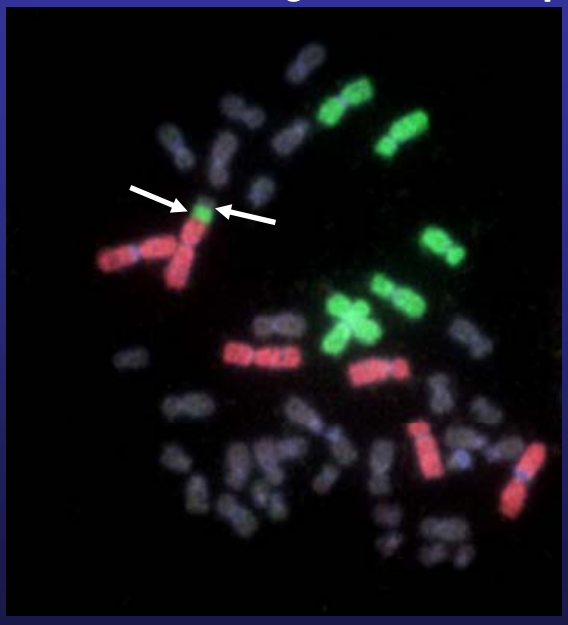


24

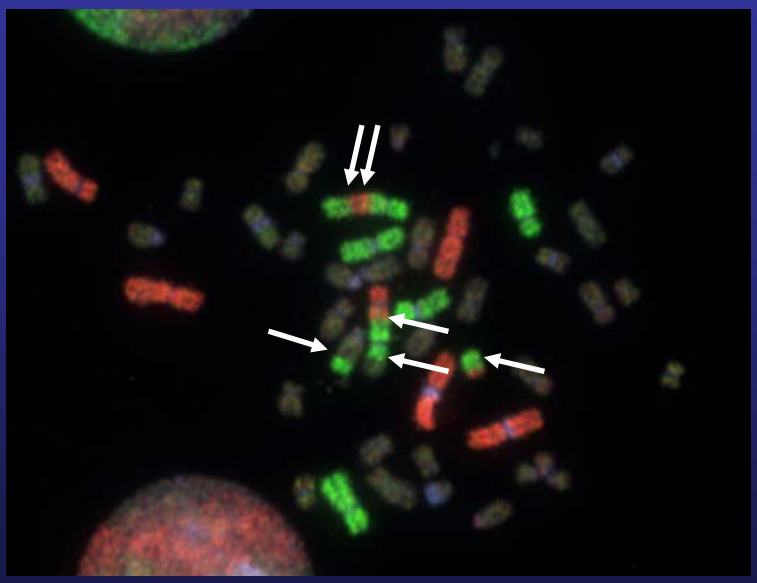
Ring chromosome plus fragment



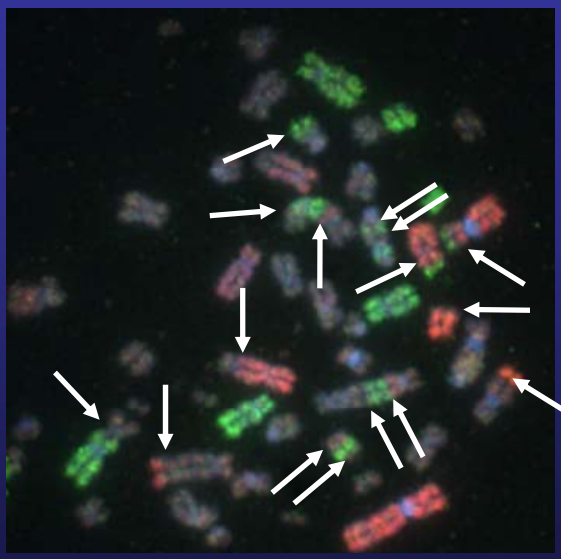
Chromosome damage can be complex



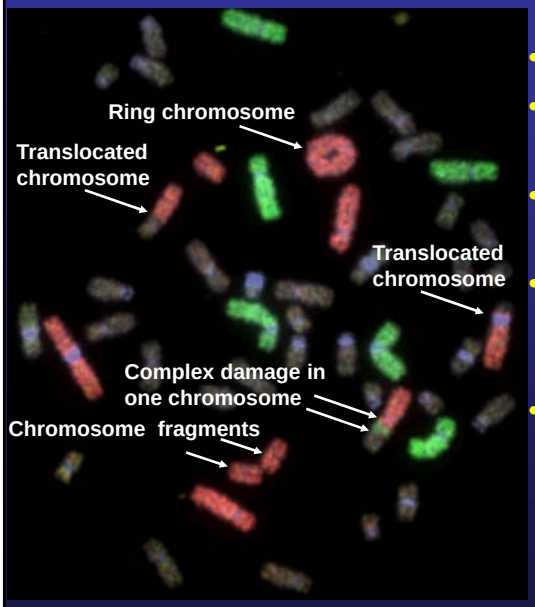
Chromosome damage can be complex



Chromosome damage can be very complex

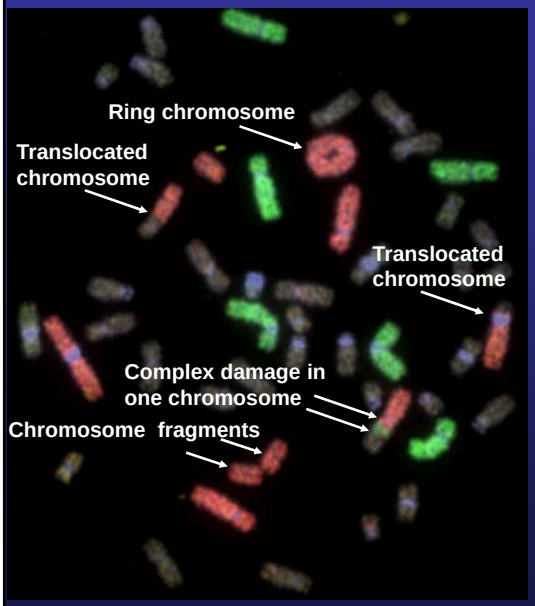


Complex Chromosome Aberrations



- Common in tumors
- Seen with some types of exposure
- Presumed high risk if cell survives
- Distribution of aberrations per cell may be important for risk assessment
- May be a marker for high-LET exposure

Complex Chromosome Aberrations



Most cells with complex aberrations:

- Fail to get through mitosis due to dicentrics, and therefore die
- Don't contribute to long-term health risks.

Cell Equivalents (CEs), Whole Genome Equivalents

Interchangeable terms.

Chromosome painting does not detect every exchange (i.e., translocation, dicentric) in a given cell.

Detectable exchanges have color junctions.

Undetectable exchanges are those that occur between chromosomes labeled in the same color.

Cell Equivalents (CEs), 1 color painting

Let p = fraction of the genome that is painted
Let q = fraction of the genome that is not painted (counterstained, usually in blue)

Then $p + q = 1$, and $p^2 + 2pq + q^2$

p^2 = unions of broken ends that were both painted

q^2 = unions of broken ends that were both unpainted

2pq = unions between one painted and one unpainted chromosome. *This is the observable fraction of all exchanges.*

Cell Equivalents (CEs), 2 color painting

With two color painting,

$p + q + r = 1$

$p^2 + 2pq + q^2 + 2pr + 2qr + r^2$

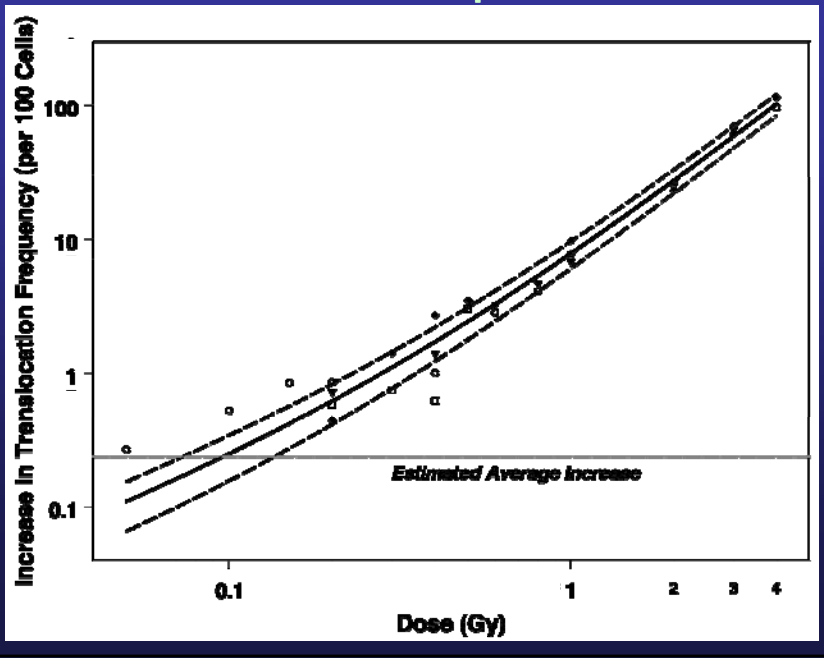
Detectable color junctions are $2pq + 2pr + 2qr$

Need to know which chromosomes are painted, and the percent of the genome they represent.

Reference: Tucker, J.D. (2010) Environmental and Molecular Mutagenesis 51:815:824.

33

^{137}Cs *in vitro* dose response curve

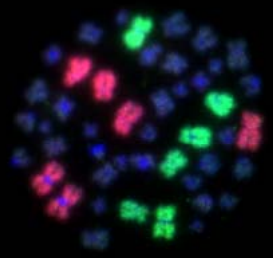


Relationships Between Environmental Exposure and Adverse Health Outcomes

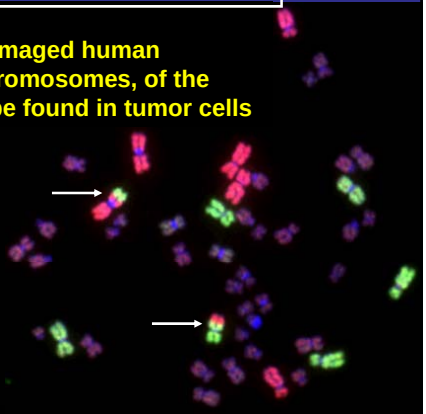
Environmental Exposure → Genetic Damage → Cancer

Environmental Exposure → Birth Defects

Normal human chromosomes



Damaged human chromosomes, of the type found in tumor cells



Chromosome Damage Can Cause Cancer

35

Why Living is Hazardous to our Health

Radiation

Chernobyl

Fukushima

Sun

Food

Drink

Oxygen

Cigarette smoke

36

Parameters for Radiation Exposure Assessment

Induction - is a measure of the relationship between exposure (dose) and some type of genetic response.

Persistence - is a measure of the longevity of induced damage.

Accumulation - is a measure of the total amount of damage in a cell, tissue, animal or person. Combines induction and persistence.

Principles for Retrospective Exposure Analysis I.

1. Selection against cells damaged by exposure does not occur or can be taken into account.
2. Translocation frequencies pre-existing in the exposed individuals should be known or be estimated from appropriate controls.
3. Clones of cytologically abnormal cells are recognizable, and their number and prevalence can be accurately measured.

Principles for Retrospective Exposure Analysis II.

4. Breaks are distributed among chromosomes in a manner that is proportional to their size.
5. The rate of exposure is known, and the effects of dose rate upon translocation frequencies are understood.
6. The influence of other confounding exposures, which may fluctuate with time, are negligible.
7. The importance of recent exposure history for determining subsequent biological responses, *i.e.*, "adaptation," is known.

Principles for Retrospective Exposure Analysis III.

8. Tumor cells are not present in the tissue being analyzed.
9. Changes in the frequency of genetic damage with age must be well characterized.
10. Susceptibility to radiation-induced chromosome aberrations is independent of age.
11. Differences between individuals with respect to the above considerations are negligible, or we can adjust for them.

To the extent these principles hold true, dosimetry using translocations can be achieved many years after exposure.

Dosimetry Confounders
Lessons Learned from Human Studies

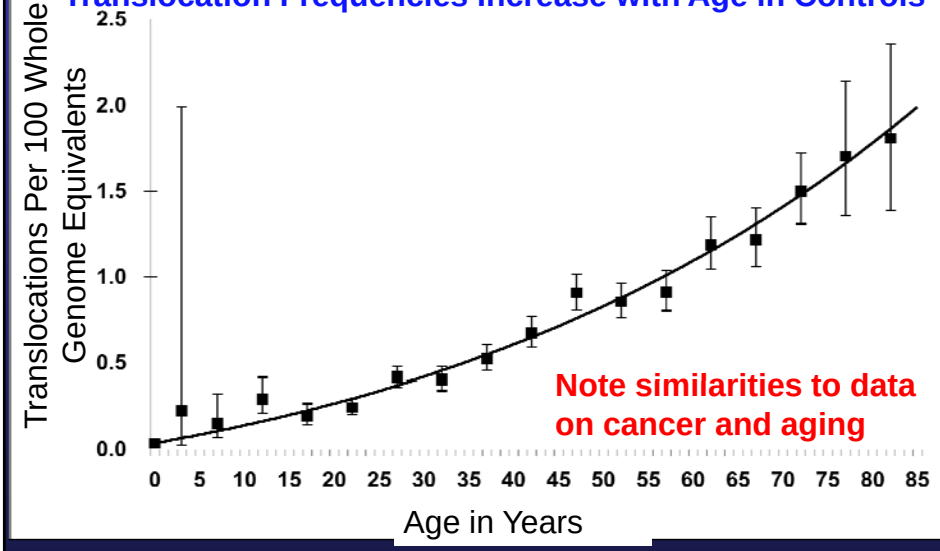
Very important
- age
- genotype
- time since exposure

Somewhat important
- smoking (depends on amount smoked)
- race (depends on the study)

Not important
- gender

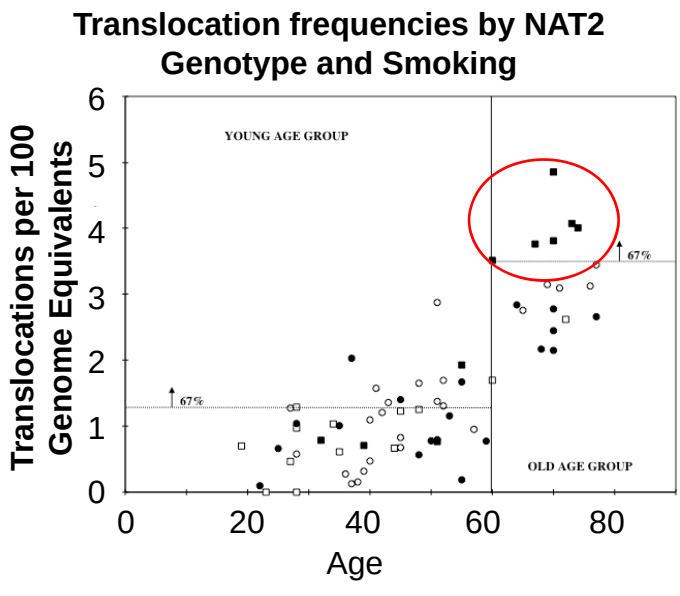
Sources of Variation: Age (Very Important)

Translocation Frequencies Increase with Age in Controls



Sigurdson *et al.*, Mutation Research (2008) 652:112-121

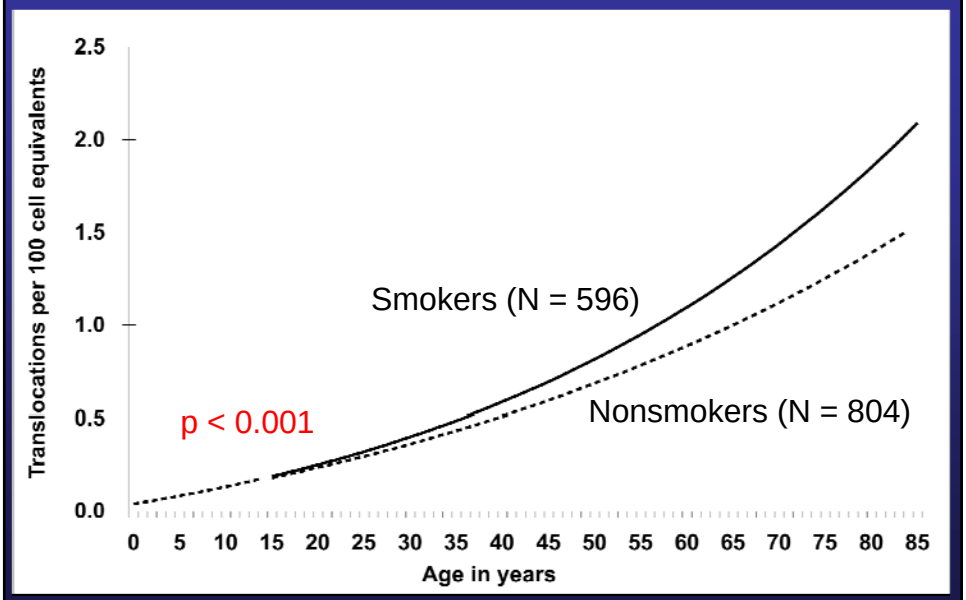
Sources of Variation: Genotypes and Smoking



Older smokers who are NAT2 rapid acetylators have significantly more translocations than everyone else.

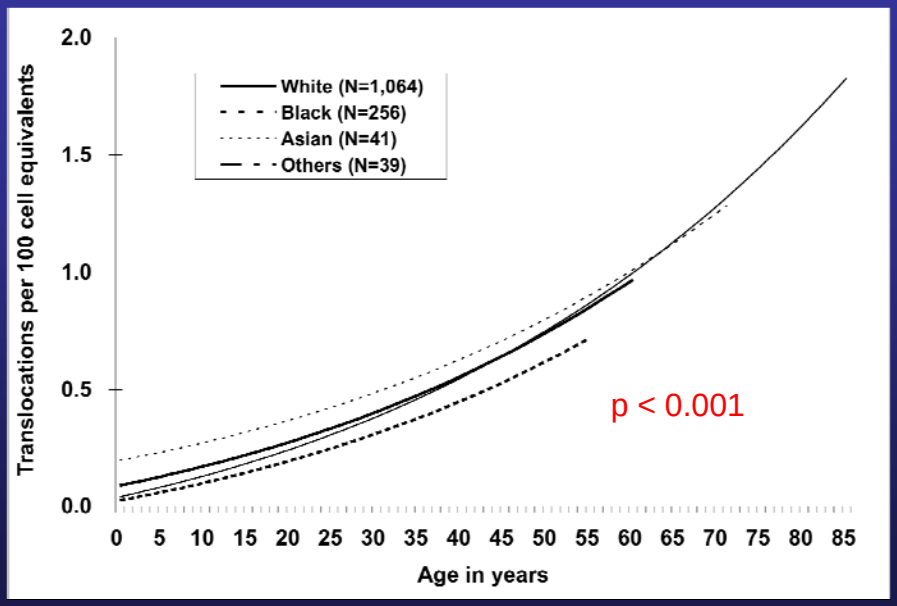
J. Pluth *et al*., (2000) Pharmacogenetics 10:311-319.

Sources of Variation: Smoking (Somewhat Important)



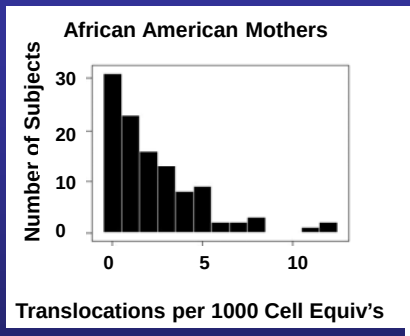
Sigurdson *et al*., Mutation Research (2008) 652:112-121

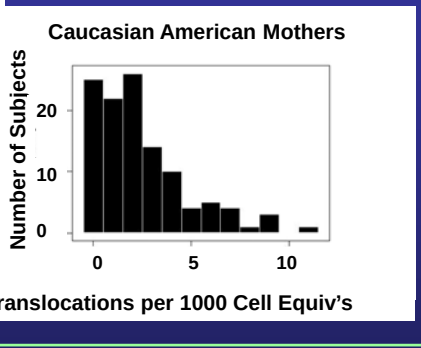
Sources of Variation: Race (Somewhat Important)



Sigurdson *et al.*, Mutation Research (2008) 652:112-121

Sources of Variation: Race (Somewhat Important)

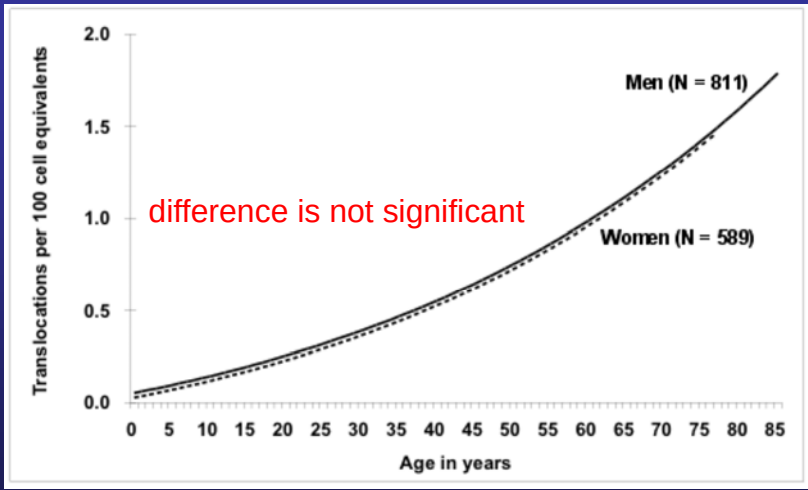




No significant difference was seen between African American and Caucasian American women at time of delivery.

Mutation Research 696:81-88 (2010)

Sources of Variation: Gender (Not Important)



Sigurdson *et al*., Mutation Research (2008) 652:112-121

Sources of Variation

Genotype: Probably quite important, but the effects of individual genes and alleles are difficult to quantify.

Smoking: Results vary from study to study. Appears to be important for estimating risk, depends on amount smoked (pack years or similar metric).

Race: Results vary. Only a few studies done. May be important for estimating risk but more work needs to be done.

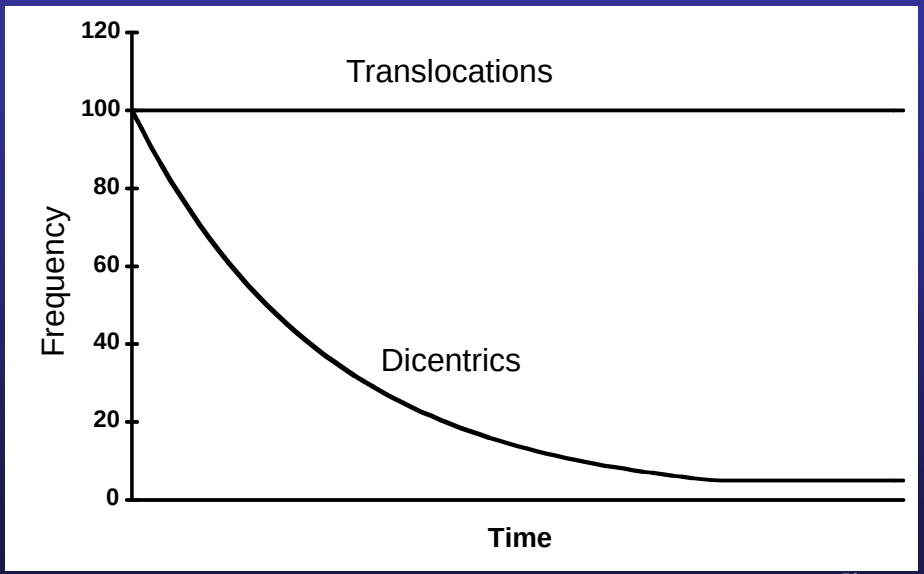
Gender: Not shown to be important.

Dosimetry Confounders – Time Since Exposure

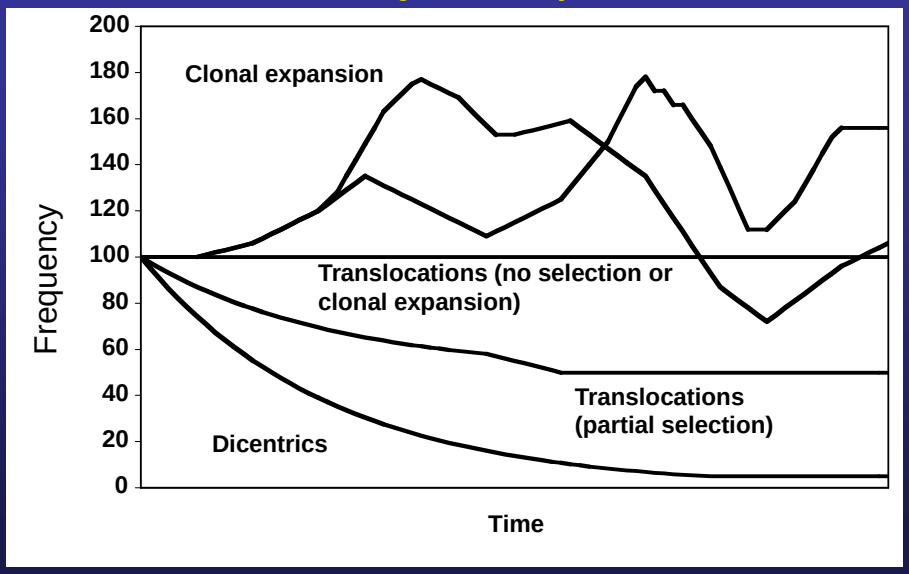
One key assumption for retrospective dosimetry is that translocations *persist*.

This makes translocations ideal for assessing temporally-displaced exposure.

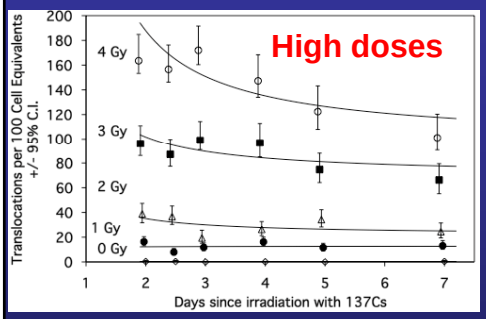
Theoretical Persistence of Translocations and Dicentrics Over Time

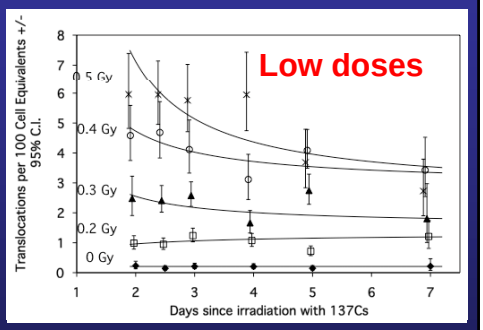


More Realistic Cytogenetic Responses Following Acute Exposure



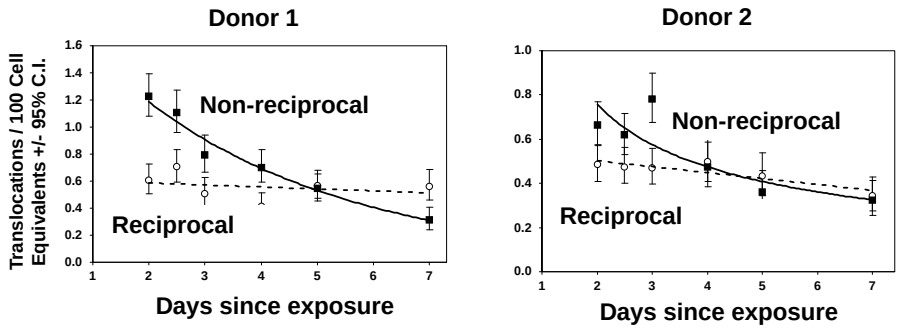
Translocation Persistence (human peripheral blood lymphocytes *in vitro*)





Environmental and Molecular Mutagenesis, 45:229-248 (2005).
Environmental and Molecular Mutagenesis, 45:249-257 (2005).

Reciprocal Translocations Show Greater Persistence than Non-reciprocal Translocations *



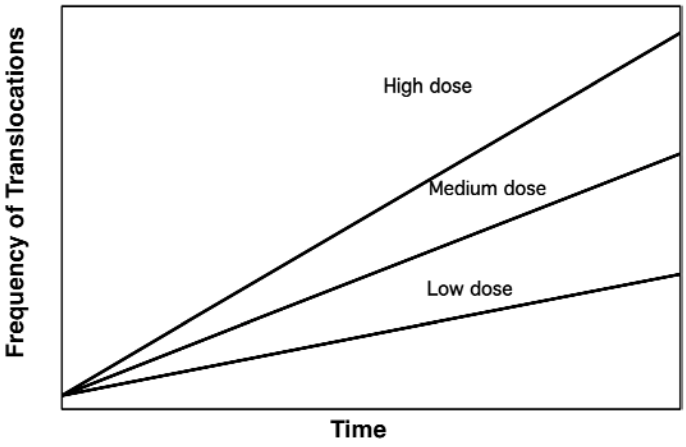
* human peripheral blood lymphocytes *in vitro*

Dosimetry Confounders – Duration of Exposure

The second key assumption is that translocations *accumulate* with exposure.

Translocations are thus ideal for assessing chronic exposures.

Theoretical Accumulation of Translocations with Time



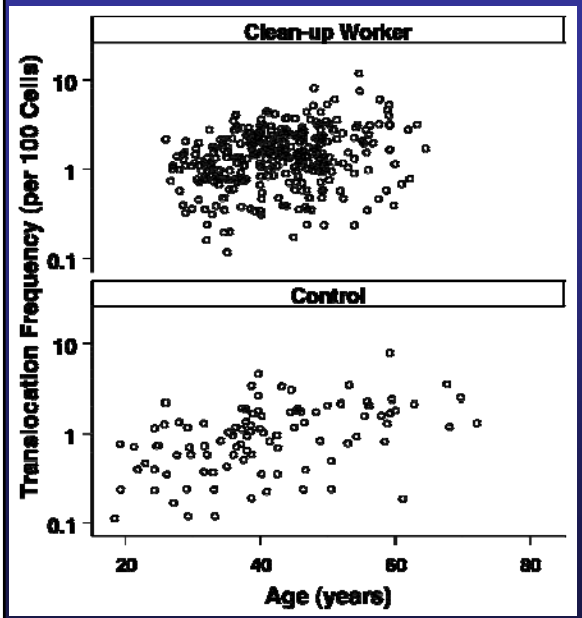
Why is Understanding Translocation Persistence so Important?

Translocations *are* lost with time but eventually reach a plateau that is greater than the original baseline

Ability to perform dosimetry is retained

Understanding the kinetics of translocation loss is important for accurate dosimetry

Chernobyl: frequencies of translocations

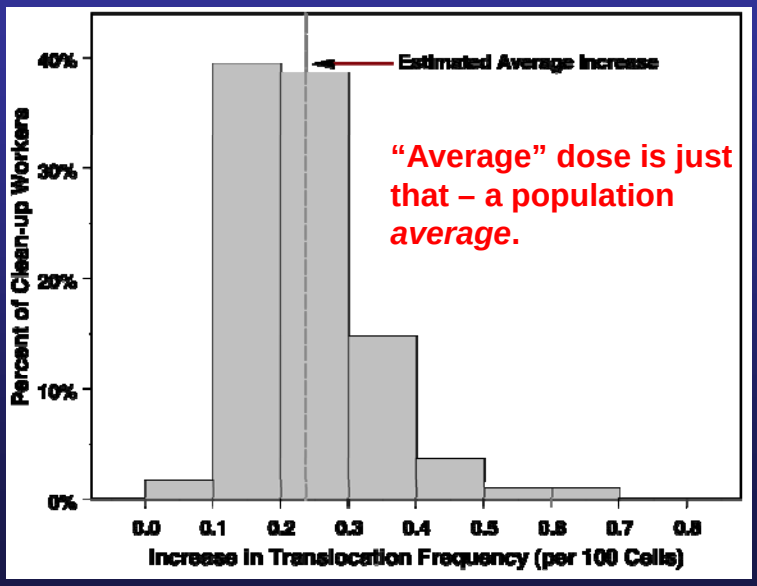


Goal: To estimate the radiation doses received by the clean-up workers.

Note considerable variation in translocation frequencies, even among subjects of similar age.

Radiation Research 158:424-442 (2002)

Chernobyl cleanup workers: Distribution of the predicted increase in translocation frequencies



"Average" dose is just that – a population *average*.

Radiation Genotoxicity from Chernobyl

Results:

1. The average dose to the clean-up workers was ~9.5 +/- 2.2 cGy, which is half the anticipated dose.

2. Translocation frequencies increase significantly with age and smoking.

3. Cytogenetic analyses have the power to detect a radiation exposure effect in the presence of confounding factors.

What about dosimetry for *individuals*?

How low can FISH biodosimetry go?

Low Dose Biodosimetry: Importance of Controls

When estimating exposure, accurate translocation counts from control(s) are essential

- same donor: *ideal, usually not possible*
- matched control: *practical, but limits detection power*
- population reference: *requires control population*

Low Dose Biodosimetry: What is the lowest detectable dose?

The answer depends on age and whether the dose is acute or chronic. Other major issues are:

- smoking status
- control sample matching
- time since exposure
- radiation quality
- level of effort expended (counting statistics)

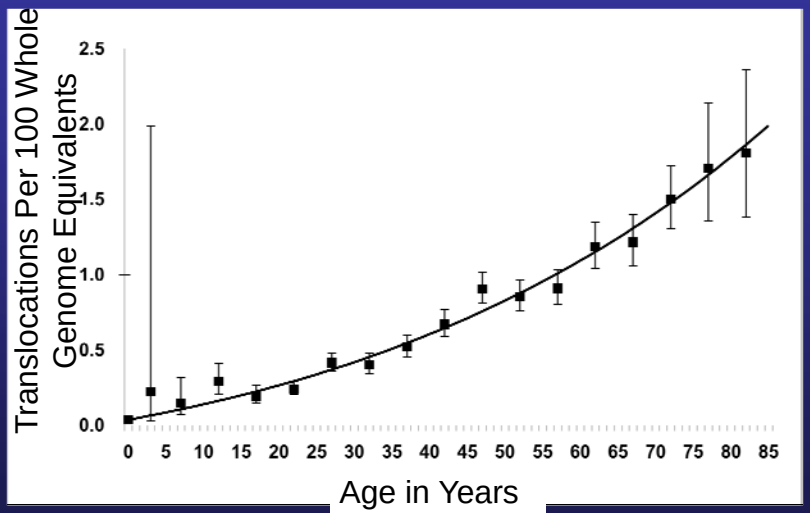
Two possible biodosimetry scenarios for an exposed individual

1. Pre-exposure sample is available
2. Pre-exposure sample is not available

Assumptions

- Calculations are for a putatively exposed individual, not for a population.
- Pre-exposure sample is not available.
- Historical data are used for controls.
- Sensitivity to ionizing radiation does not change with age.

Translocations and Age in Unexposed People



Sigurdson *et al*., Mutation Research (2008) 652:112-121

Summary of the Sigurdson *et al*. Data

N = 1933 subjects.

All were apparently normal, healthy people.

None had been treated with radiation or chemotherapy.

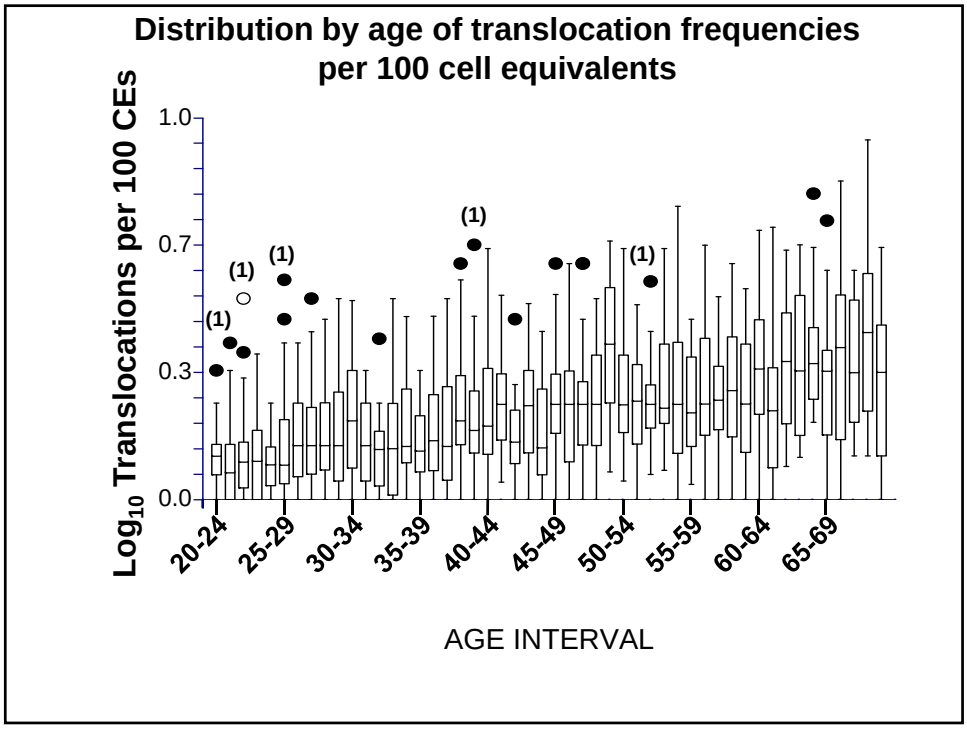
"Control" subjects in other studies.

age interval	N	mean per 100 CEs	stdev
0 - 4	299	0.04	0.09
5 - 9	38	0.16	0.25
10 - 14	29	0.22	0.26
15 - 19	65	0.21	0.25
20 - 24	191	0.31	0.34
25 - 29	177	0.53	0.54
30 - 34	138	0.53	0.51
35 - 39	154	0.65	0.61
40 - 44	141	0.74	0.61
45 - 49	152	1.06	0.83
50 - 54	140	1.02	0.80
55 - 59	112	1.00	0.75
60 - 64	87	1.41	1.09
65 - 69	102	1.49	1.27
70 - 74	61	1.66	1.02
75 - 79	22	1.86	1.16
80 - 85	25	2.17	1.47

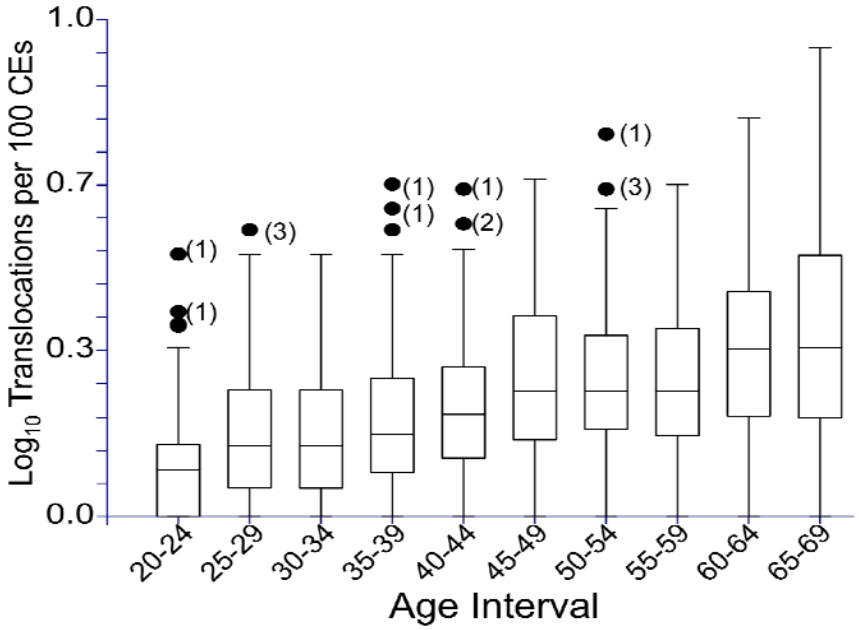
Step 1 in our analyses

Using the data of Sigurdson *et al*. (2008), we calculated the number of translocations per 100 whole-genome cell equivalents (CEs) in one and five-year age intervals from birth to 80+.

Outliers were removed to satisfy the assumptions of the regression analyses.



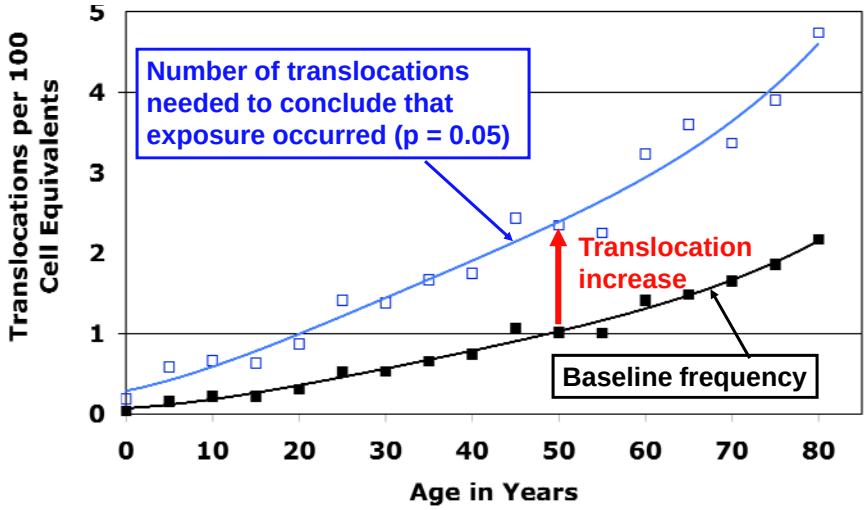
Distribution of translocation frequencies per 100 cell equivalents in 5-year age intervals



Step 2 in our analyses

For each 5-year age interval, we calculated the number of translocations per 100 CEs needed in a putatively exposed individual to conclude that a significant increase had occurred with probabilities p = 0.05, p = 0.01, and p = 0.001.

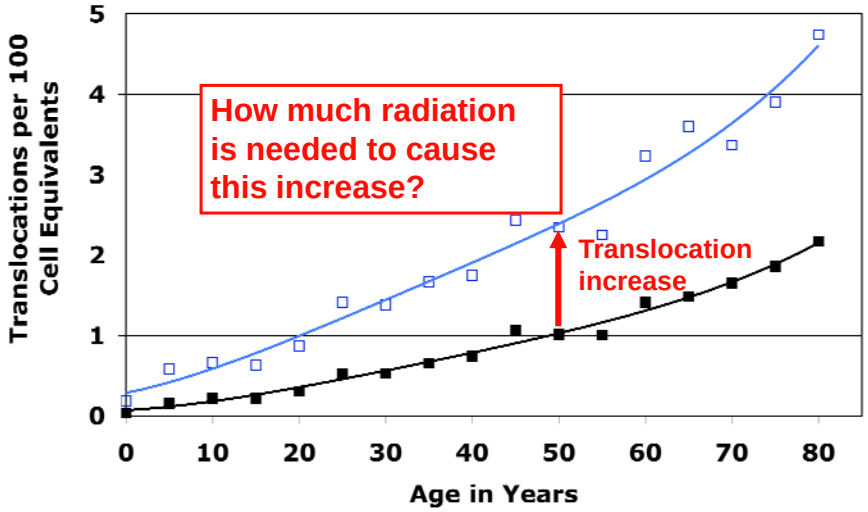
Increase Needed in Translocations to Detect Exposure in an Individual



Step 3 in our analyses

For each 5-year age interval we calculated the dose that is needed to induce the minimum number of translocations required for a statistically significant increase.

Increase Needed in Translocations to Detect Exposure in an Individual



Step 3, concluded

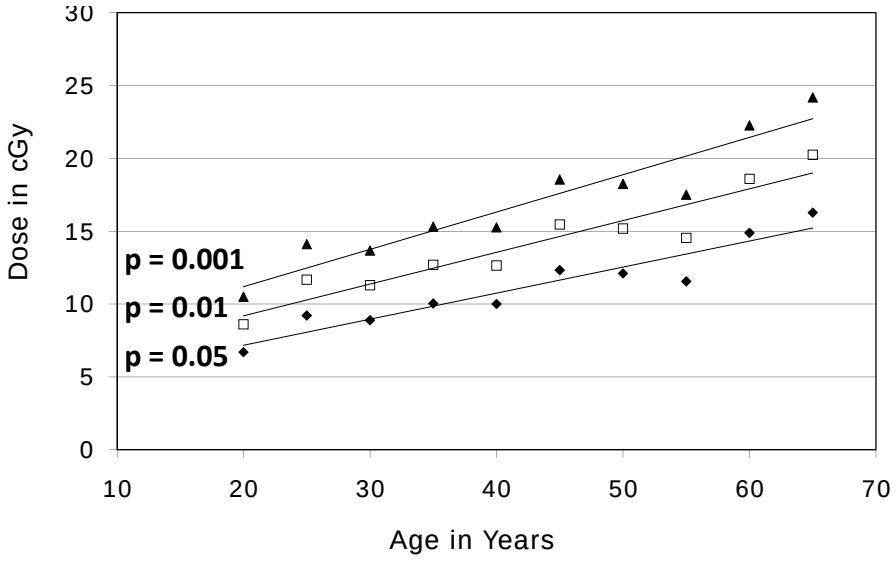
We used the dose response curve generated *in vitro* with ^{137}Cs (Jones *et al*., 2002, *Rad. Res.* 158, 424-442).

Number of translocations per CE =
$k + 0.019D + 0.0597D^2$

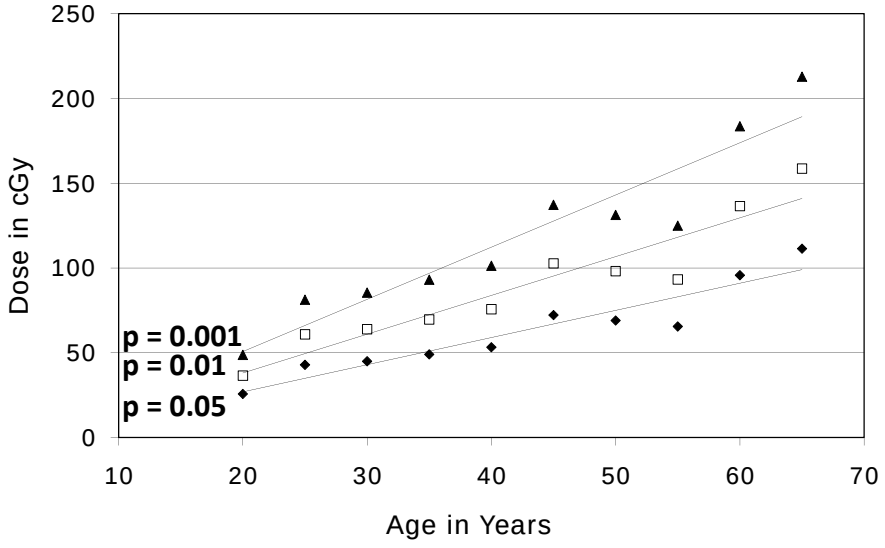
where:

k is the baseline translocation frequency
D is the dose in Gy

Hypothetical minimum detectable acute dose by age in adults



Hypothetical minimum detectable chronic dose by age in adults



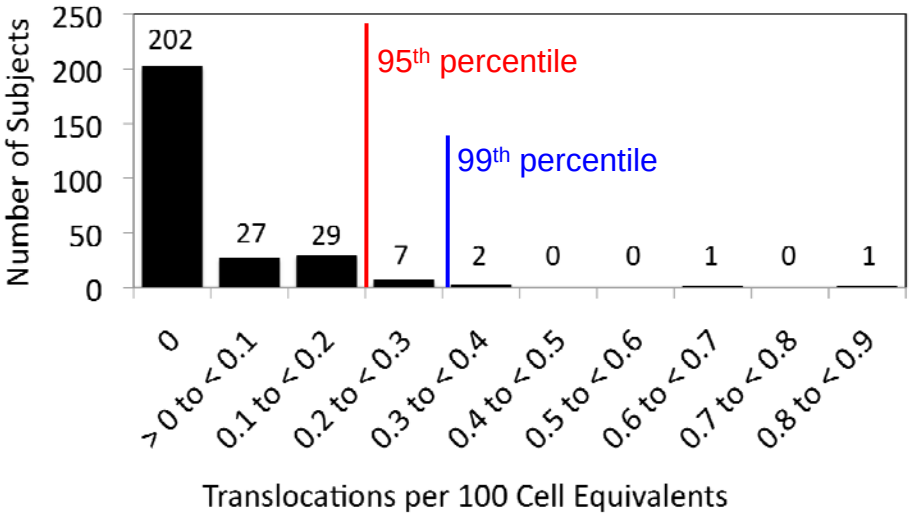
Parameters of the regression fits

Table 2. Parameters of the regression fits.

		slope			intercept			
Exposure duration	Significance level evaluated	coefficient	standard error	p-value	coefficient	standard error	p-value	Adjusted R-squared
Acute	0.05	0.179	0.022	< 0.0001	3.592	0.969	0.006	0.88
	0.01	0.218	0.026	< 0.0001	4.842	1.176	0.004	0.88
	0.001	0.256	0.031	< 0.0001	6.073	1.373	0.002	0.88
Chronic	0.05	1.591	0.265	< 0.0001	-7.55	11.87	0.54	0.80
	0.01	2.270	0.377	< 0.0001	-11.03	16.92	0.53	0.80
	0.001	3.055	0.508	< 0.0001	-15.38	22.77	0.52	0.80

Tucker, J.D. and Luckinbill, L.S. (2011) Radiation Res. 175:631-7

Translocation frequencies in 269 newborns



Summary – lowest detectable dose

The dose needed to cause a statistically significant elevation in translocations in an individual increases linearly with age

Acute exposure: 0.18 cGy per year of age

Chronic exposure: 1.59 cGy per year of age

Detecting a given level of exposure is more challenging in older than in younger people.

Summary of FISH Radiation Biodosimetry

Translocations are the preferred endpoint

- FISH painting is the best method
- fast and reliable
- time since exposure important but not essential
- sensitive enough to detect low doses
  - ✓ populations
  - ✓ individuals

Major confounders:

- age
- cigarette smoking
- possibly genotype (requires more research)

Thank you